Biochemical Pharmacology, Vol. 27, pp. 1347–1351.

0006-2952/78/0501-1347/$02.00/0

EFFECTS OF MORPHINE ON ISOENZYMES OF PYRUVATE KINASE AND TYROSINE AMINOTRANSFERASE IN RAT

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(*Received* 15 *September* 1977; *accepted* 24 *November* 1977)

Abstract—The acute and chronic effects of morphine on isoenzymes of pyruvate kinase and tyrosine aminotransferase in rat liver have been studied *in vivo*. Acute administration of morphine inhibits the L-type pyruvate kinase but increases forms II, III and IV of tyrosine aminotransferase in rats which have received a single intraperitoneal injection of morphine 6 hr prior to sacrifice. However both effects are lost after 24 hr. Morphine starts to regain its stimulatory effect on tyrosine aminotransferase at the hr 48 and reaches a maximum at the hr 72. Chronic morphinization leads to similar changes in the total activities of pyruvate kinase and tyrosine aminotransferase but the change in isoenzyme pattern of tyrosine aminotransferase concerns only forms II and III. Results from this study support the hydrocortisone-like property of morphine and indicate that morphine may enhance gluconeogenesis *in vivo*.

It has been demonstrated previously that morphine acts as a pseudo-hormone, participating in the regulation of cellular metabolism [1]. This is attributed to its structural similarities to both adrenaline and steroids. The evidence that morphine does mimic many of the effects of adrenaline is well documented [2, 3]. However the hydrocortisone-like effect of morphine remains to be established. Since hydrocortisone is the major controlling factor in the regulation of the activity of tyrosine aminotransferase, and Weber has proposed that steroids play a very important role in the control of pyruvate kinase synthesis, it is of interest to investigate if morphine can exert the same effect on these enzymes [4, 5]. Both of these enzymes have been shown to exist in multiple molecular forms [6–11]. Earlier studies by Kenney [6, 7], Oliver [8, 9] and Pitot [10] have indicated that rat hepatic tyrosine aminotransferase exists in four distinct forms and they are under the regulation of different hormones. Pyruvate kinase from rat liver has also been shown to exist in two forms which show quite different regulatory behaviour [11]. The liver-type (L-type) is under the control of hormones while the muscle-type (M-type) is unresponsive to hormonal treatment. The present paper attempts to find out whether any of these isoenzymes are under the influence of morphine in order to get insight into the mechanism of morphine action on metabolism.

MATERIAL AND METHODS

Animals. Male albino rats of approximately 220 g were used and were housed in a light–dark regulated room with 12 hr artificial lighting per day. They were allowed free access to food and water. They were randomized into nine groups for different treatments. The first group received saline injection acting as normal control. The second and third group received morphine sulphate (30 mg/kg body wt) intraperitoneally (i.p.) and were killed 6 and 24 hr after administration. The fourth and fifth group received daily injections of 30 mg/kg morphine sulphate intraperitoneally and were killed 48 and 72 hr after the first administration. The sixth group received chronic injection of morphine according to the method of Gourley [12] for 17 days. The seventh group received single i.p. injection of adrenaline (2.5 mg/kg) and were killed 30 min, 1, 1½, 2, 2½ and 6 hr after administration. The eighth group received a single i.p. injection of hydrocortisone 21-sodium succinate (100 mg/kg) and were killed 6 and 18 hr after the treatment. The ninth group received three daily injections of 100 mg/kg hydrocortisone 21-sodium succinate intraperitoneally and were sacrificed 24 hr after the last administration.

Chemicals. All chemicals used were of analytical grade. Phosphoenolpyruvate (tricyclohexylamine salt), lactic dehydrogenase, NADH, ADP, pyridoxal phosphate, dithiothreitol, sodium diethyldithiocarbamate, α-ketoglutarate, hydrocortisone (21-sodium succinate) and adrenaline were obtained from Sigma Chemical Co., St. Louis, MO. L(−)-Tyrosine was obtained from E. Merck. Hydroxyapatite was prepared by the modified method of Tiselius [13].

Preparation of enzyme extracts. Animals were sacrificed between 9.30–10.30 a.m. by decapitation. The liver was homogenized in 4 vol. of 0.1 M potassium phosphate buffer pH 7.6 containing 4 mM pyridoxal phosphate and 1 mM dithiothreitol. Homogenization was standardized by using ten strokes of Potter–Elvehjem homogenizer. The homogenate was centrifuged at 160,000 *g* for 30 min at 4° and the supernatant was collected. 2 ml of the supernatant was applied to a hydroxyapatite column (1.5 cm diameter × 10 cm) which had been equilibrated with 0.1 M potassium phosphate buffer pH 6.9 contain-

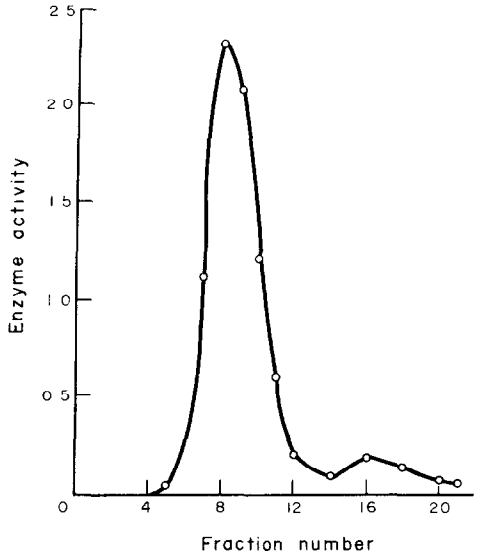

Fig. 1. Hydroxyapatite chromatographic elution pattern of hepatic pyruvate kinase. The enzyme activity is expressed as μmoles pyruvate formed/fraction/min at 37°. The first isoenzyme eluted off at a phosphate concentration of 0.18 M is the L-type while the second isoenzyme eluted off at a phosphate concentration of 0.24 M is the M-type.

ing 1 mM dithiothreitol. After washing the column with the initial buffer, elution was started by a linear gradient of potassium phosphate buffer (pH 6.9 from 0.1 M to 0.5 M) containing 1 mM dithiothreitol. Two ml fractions were collected for both pyruvate kinase and tyrosine aminotransferase determinations.

Enzyme assays. Pyruvate kinase activity was assayed by the method of Bucher and Pfleiderer [14]. Tyrosine aminotransferase activity was assayed by the method of Iwasaki and Pitot [10].

RESULTS

Isoenzymes of pyruvate kinase were separated by hydroxyapatite column into two forms as shown in Fig. 1, while those of tyrosine aminotransferase were separated into four forms similar to those reported by Yeung and Yeung [15].

Effect of acute administration of morphine on hepatic pyruvate kinase and tyrosine aminotransferase. The acute effects of morphine on hepatic pyruvate kinase and tyrosine aminotransferase were rather time-dependent. The first group of animals which were killed 6 hr after morphine administration had a very abrupt increase in tyrosine aminotransferase activity while pyruvate kinase decreased only by 26 per cent (Table 1). As the duration of exposure to morphine was prolonged to 24 hr, both pyruvate kinase and tyrosine aminotransferase reverted to normal. Rats that had received daily injection of morphine (30 mg/kg body wt) for 2 days and killed 48 hr after the first administration had an increase in tyrosine aminotransferase (elevation being 32 per cent) but their pyruvate kinase activity still remained unchanged (Table 2). Rats treated with morphine for 3 days showed changes both in pyruvate kinase and tyrosine aminotransferase activities—inhibition in pyruvate kinase being 50 per cent while elevation in tyrosine aminotransferase being 48 per cent (Table 2).

Isoenzyme studies reveal that of the four forms of tyrosine aminotransferase, acute morphine administration increased the levels of forms II, III and IV while form I was unaffected. With regard to pyruvate kinase activity, morphine only decreased the L-type but had no effect on the M-type.

Effect of chronic morphinization on hepatic pyruvate kinase and tyrosine aminotransferase. Chronic morphinization tended to decrease hepatic pyruvate kinase by 55 per cent and increased tyrosine aminotransferase activity by 44 per cent (Table 1). However the effect of chronic morphinization was slightly different from acute administration in that the increase in tyrosine aminotransferase activity concerned mainly with forms II and III. Form IV which was increased most significantly in acute administration remained unchanged after chronic morphinization.

Effect of adrenaline and hydrocortisone administration on hepatic pyruvate kinase. Our results showed that intraperitoneal injection of adrenaline into rats did lower the pyruvate kinase activity but the effect was rather time-dependent. Significant inhibition occurred 2 hr after the injection but the inhibitory effect was lost when the duration of exposure to the hormone was prolonged to 6 hr. Again the effect of adrenaline was only on the L-type (Table 3). However i.p. injection of hydrocortisone

Table 1. Acute and chronic effects of morphine on isoenzymes of pyruvate kinase and tyrosine aminotransferase

	Enzyme activity, mean ± S.E.M.					
	Acute administration, 6 hr			Chronic morphinization		
Types of enzymes	Normal* (6)	Morphinized* (10)	% Change	Normal* (4)	Chronically morphinized* (6)	% Change
Pyruvate kinase total activity	32.62 ± 2.31	24.25 ± 1.77	−26% (P < 0.02)	33.93 ± 4.54	15.33 ± 1.24	−55% (P < 0.01)
L-type	29.70 ± 2.71	21.50 ± 1.55	−27% (P < 0.02)	31.27 ± 3.13	12.54 ± 0.66	−59% (P < 0.001)
M-type	2.92 ± 0.26	2.75 ± 0.30	−6% (N.S.)	2.66 ± 0.19	2.79 ± 0.18	+6% (N.S.)
Tyrosine aminotransferase total activity	99.72 ± 8.50	184.40 ± 25.61	+85% (P < 0.01)	122.89 ± 7.24	177.21 ± 15.01	+44% (P < 0.02)
Form I	3.39 ± 0.41	3.45 ± 0.14	+3% (N.S.)	5.09 ± 0.84	6.09 ± 0.54	+20% (N.S)
Form II	76.48 ± 10.20	125.09 ± 18.01	+64% (P < 0.05)	96.29 ± 4.51	138.13 ± 12.43	+43% (P ≈ 0.02)
Form III	16.69 ± 1.61	43.53 ± 6.31	+221% (P < 0.001)	15.96 ± 3.49	26.46 ± 2.01	+67% (P < 0.05)
Form IV	3.16 ± 0.50	12.33 ± 3.02	+296% (P < 0.01)	5.55 ± 0.56	6.53 ± 0.58	+18% (N.S.)

Pyruvate kinase activity is expressed as μmoles pyruvate formed per gram wet tissue per minute. Tyrosine aminotransferase activity is expressed as μmoles *p*-hydroxyphenylpyruvate formed/g wet tissue/hr. Reactions are carried out at 37°. There is no detectable change in either the total activities of isoenzyme patterns of pyruvate kinase and tyrosine aminotransferase 24 hr after acute administration.

* Numbers in parenthesis indicate number of rats used.

Table 2. Acute effect of morphine, 48 h and 72 h after administration on isoenzymes of pyruvate kinase and tyrosine aminotransferase

Types of enzymes	Enzyme activity, mean ± S.E.M. 48 hr after administration Normal* (4)	Morphinized* (4)	% Change	72 hr after administration Normal* (4)	Morphinized* (4)	% Change
Pyruvate kinase total activity	25.51 ± 1.38	23.58 ± 1.17	−8% (N.S.)	27.18 ± 1.26	13.62 ± 0.97	−50% (P < 0.001)
L-type	23.34 ± 1.21	21.53 ± 1.41	−8% (N.S.)	24.67 ± 1.66	10.86 ± 1.50	−56% (P ≈ 0.001)
M-type	2.17 ± 0.18	2.05 ± 0.22	−6% (N.S.)	2.51 ± 0.31	2.76 ± 0.19	+11% (N.S.)
Tyrosine aminotransferase total activity	87.68 ± 6.90	115.71 ± 8.04	+32% (P < 0.05)	96.58 ± 4.27	143.44 ± 7.74	+48% (P < 0.001)
Form I	4.39 ± 0.80	4.47 ± 0.91	+2% (N.S.)	4.10 ± 0.47	4.54 ± 0.48	+11% (N.S.)
Form II	70.26 ± 2.91	85.58 ± 4.80	+22% (P < 0.05)	61.16 ± 6.10	84.46 ± 7.02	+38% (P < 0.05)
Form III	9.19 ± 1.19	17.57 ± 2.58	+91% (P < 0.05)	21.75 ± 2.32	36.10 ± 3.74	+66% (P < 0.02)
Form IV	3.84 ± 0.70	8.09 ± 0.61	+110% (P < 0.01)	9.57 ± 1.05	18.32 ± 2.49	+92% (P = 0.02)

Pyruvate kinase activity is expressed as μmoles pyruvate formed/g wet tissue/min. Tyrosine aminotransferase activity is expressed as μmoles *p*-hydroxyphenylpyruvate formed/g wet tissue/hr. Reactions are carried out at 37°.
* Numbers in parentheses indicate number of rats used.

into rats either by single injection or after three daily injections failed to produce any effect on pyruvate kinase.

DISCUSSION

It is well documented that pyruvate kinase is under dietary and hormonal control and shows regulatory properties especially suited for an opposing control of glucose degradation and synthesis. Two interconvertible forms of pyruvate kinase regulated by cyclic AMP-dependent protein kinase have been reported by different groups of investigators [16–19]. The interconversion of the multiple forms of tyrosine aminotransferase is under the influence of glucagon, adrenaline, cyclic AMP and hydrocortisone. The results presented in this paper demonstrate that acute morphinization inhibits pyruvate kinase activity but stimulates tyrosine aminotransferase activity (Table 1). The inhibitory effect of morphine on pyruvate kinase can be explained by the interconversion of the active dephosphorylated form into its inactive phosphorylated form through an elevation of hepatic cyclic AMP concentration. Such a change in the cyclic nucleotide concentration may be brought about by activation of the adenyl cyclase activity in a manner analogous to that of adrenaline. As morphine possesses structural similarity to that of adrenaline, it may act on the adrenaline receptor site. The present study shows that of the two isoenzymes of pyruvate kinase, adrenaline only affects the L-type (Table 3) and this is in agreement with the finding of Tanaka [11]. Furthermore, morphine also exerts its effect only on the L-type (Table 1) suggesting that its mechanism of action may indeed be very similar to that of adrenaline.

Cyclic AMP-dependent phosphorylation of pyruvate kinase is a rather "short-term" effect because the inactive phosphorylated pyruvate kinase can be converted to the active form by means of a histone phosphate present in the soluble fraction as suggested by Titanji [20]. This can be demonstrated from the "time-dependent" effect of adrenaline on pyruvate kinase (Table 3). Adrenaline starts to exert its effect 2 hr after i.p. injection but its inhibitory effect is lost when the time of exposure is prolonged to 6 hr. Most probably at the sixth hr after adrenaline administration, the elevated cyclic AMP has undergone degradation and the phosphorylated pyruvate kinase has been converted to the dephosphorylated form by histone phosphatase. Similar results have been obtained with morphine which is much less potent than adrenaline and therefore takes longer time to show its effect. Morphine starts to inhibit pyruvate kinase at the sixth hr but its effect

Table 3. Effect of adrenaline and hydrocortisone administration on hepatic pyruvate kinase

Time after administration	Enzyme activity, mean ± S.E.M. Total	L-type	M-type	% Change
+ Saline control (6)*	29.33 ± 1.76	26.75 ± 1.78	2.58 ± 0.21	—
+ Adrenaline				
½ hr (4)*	26.88 ± 1.82	—	—	−8% (N.S.)
1 hr (4)*	32.91 ± 2.17	—	—	+9% (N.S.)
1½ hr (4)*	31.38 ± 2.36	—	—	+7% (N.S.)
2 hr (4)*	15.26 ± 1.07	12.79 ± 1.04	2.47 ± 0.25	−48% (P < 0.001)
2½ hr (4)*	15.82 ± 1.54	13.34 ± 1.20	2.48 ± 0.19	−46% (P < 0.01)
6 hr (4)*	30.71 ± 2.93	—	—	+5% (N.S.)
+ Hydrocortisone				
6 hr (4)*	32.58 ± 2.86	—	—	+11% (N.S.)
18 hr (4)*	25.81 ± 1.53	—	—	−12% (N.S.)
72 hr (4)*	31.67 ± 2.57	—	—	+8% (N.S.)

Pyruvate kinase activity is expressed as μmoles pyruvate formed/g wet tissue/min at 37°.
* Numbers in parentheses indicate number of rats used.

is lost when the time of exposure to the drug is prolonged (Table 2). Thus no appreciable effect of morphine on the enzyme can be demonstrated at hr 24 and 48, after drug administration. However the inhibitory effect of morphine on hepatic pyruvate kinase 72 hr after administration (Table 2) cannot be due to its adrenaline-like or hydrocortisone-like properties since adrenaline-like effect is rather "short term" and hydrocortisone itself when given i.p. fails to exert any effect on hepatic pyruvate kinase (Table 3). Hence it is not certain by what mechanism morphine can cause a decrease in hepatic pyruvate kinase under such regime.

The demonstration of an increase in tyrosine aminotransferase activity 6 hr after morphine administration agrees with the finding reported by Ferri *et al.* [21]. However this increase cannot be attributed to increased cyclic AMP alone since cyclic AMP induces predominantly form II [12] and to a smaller extent form III [15] while hydrocortisone induces forms II, III and IV [15, 22]. Besides, Nikodijevic and Maickel have reported that single dose of morphine generally stimulates adrenal cortical secretion in rat [23]. The plasma corticosterone level becomes elevated within 30 min after the i.p. injection of morphine and remains elevated for 6 hr but returns to normal after 24 hr. Of the four forms of tyrosine aminotransferase, morphine increases the activity of forms II, III and IV indicating that its effect on tyrosine aminotransferase is very similar to that of hydrocortisone. Thus the stimulatory effect of morphine 6 hr after administration can be attributed to a combined action of elevated cyclic AMP and elevated plasma corticosterone level. Furthermore the disappearance of enzymatic stimulation after 24 hr supports our proposal because at that instance the plasma corticosterone level has reverted to normal level.

However as is evidenced from Table 2 morphine regains its stimulatory effect on tyrosine aminotransferase when the duration of exposure to the drug is prolonged to 48 hr. At this stage, the plasma corticosterone level has been normalized. The increase in tyrosine aminotransferase is most likely due to morphine itself acting as a pseudohormone. Isoenzyme study reveals that the increase in tyrosine aminotransferase is a result of the elevation of forms II, III and IV. The stimulatory effect of morphine on tyrosine aminotransferase attains a higher level when three daily injections of morphine are given, the elevation being 48 per cent (Table 2). The change in isoenzyme patterns is rather similar to those after 48 hr administration. These findings can be taken to suggest that morphine is acting like hydrocortisone. Morphine and steroid hormones are both derivatives of perhydrophenanthrene. *In vitro* studies show that morphine interferes with corticosteroid binding capacity and competes for steroid binding sites in rat [24, 25]. Thus morphine can mimic the hydrocortisone-induced enzyme synthesis.

In previous studies, it has been shown that those changes brought about by acute administration may be different or less marked in rats chronically treated with morphine [2, 3]. In the present study we have found that chronic morphinization also leads to similar changes in the total activities of hepatic pyruvate kinase and tyrosine aminotransferase. However the change in isoenzyme patterns of tyrosine aminotransferase is different from those observed after acute treatment. This difference may be the consequence of the development of tolerance to morphine which causes cellular adaptation as reported by many workers [26–31] thereby setting up a new hormonal balance for regulation of metabolism. In one of our studies (unpublished observation) we have found that hepatic cyclic AMP level after chronic morphinization has been increased by 70 per cent. This increase in hepatic cyclic AMP may maintain a higher proportion of pyruvate kinase in the inactive phosphorylated form and brings about an increase in the synthesis of hepatic tyrosine aminotransferase.

Contrary to our findings, Ferri *et al.* [21] reported that chronic morphinization has no effect on tyrosine aminotransferase. Careful inspection of the experimental procedure shows that rats of different sex were used in the two studies. Ferri *et al.* [21] used female rats of 150 ± 20 g whereas we used male rats of 220 ± 30 g. We observed that tyrosine aminotransferase activity fluctuates in female rats which may be a consequence of the estrus cycle. Possibly of more significance is the dosage and mode of morphine administration. In the study of Ferri *et al.* [21] chronically morphinized rats were treated with morphine 20 mg/kg/subcutaneously (s.c.) daily for 18 days with no increase in the dosage whereas in our studies chronic morphinization was done according to Gourley [12] where increasing dose of morphine was given twice a day until a dose of 220 mg/kg was reached on day 17. These differences in experimental procedure may account for the discrepancy in result from chronically morphinized animals.

Recent publications have shown that morphine affects the activities of hepatic gluconeogenic enzymes and epididymal lipase activity [2, 3]. The latter directly contributes to precursor for gluconeogenesis. The present data also indicates that gluconeogenesis may indeed be enhanced *in vivo* as suppressed activity of pyruvate kinase coupled with elevated level of tyrosine aminotransferase thereby furnishing more carbon skeleton from both tyrosine and phenylalanine are conducive for glucose synthesis.

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